

FREQUENCY OF *ESCHERICHIA COLI* ISOLATES FROM PIGS SUFFERING FROM DIFFERENT MANIFESTATIONS OF "POSTWEANING COLI COMPLEX" (PWCC) IN AGRICULTURAL HUNGARY / ROMANIA / SERBIA

DOCIC M and BILKEI G

Bilkei Consulting, Dübendorf, Switzerland

(Received 11. November 2002)

Gross postmortem pathologic-anatomical examination and polymerase chain reaction analyses for fimbriae (F), heat-stable (ST) and heat-labile (LT) enterotoxin and verotoxin (Shiga-like toxin (SLT) type II) were performed for 351 weaned piglets originating from units in agricultural Hungary / Romania / Serbia. The majority of isolates (94,87%) carrying enterotoxin genes also carried genes for one of the fimbrial adhesins. The predominant genotypes were F4 LT STb and F18 Sta STb SLT respectively. This genetic combination may be explained by regional clonal expansion. Sixty-nine (20%) animals were diagnosed with postweaning diarrhea, thirty five (10%) with edema disease, 240 (68%) with postweaning wasting and 7 piglets (2%) exhibited hemorrhagic gastroenteritis. Postweaning wasting was the major cause of losses.

Key words: Swine, Escherichia coli, weaning, fimbrial adhesins, genotypes, diarrhea, edema disease, wasting, hemorrhagic gastroenteritis

INTRODUCTION

Postweaning problems caused by *Escherichia coli* (in Eastern Europe known as "postweaning coli complex", PWCC, Bilkei *et al.*, 1995) are important causes of poor health and reduced economic performance of weaned piglets. Although new production systems using early weaning and multisided production, have efficiently controlled and eliminated postweaning problems in Western Europe (Bölcskei *et al.*, 1995), in some parts of Eastern Europe, especially in the countryside of Hungary, Romania and Serbia, they still remain a constant challenge for the consulting veterinarian (Bilkei *et al.*, 1996b).

Weaning is one of the most stressful challenges in the life of piglets (Bölcskei *et al.*, 1995). Local immunity provided by the milk suddenly vanishes and the gut's physiology has to adapt quickly to a different type of nutrition. Colibacillosis is a major cause of illness and death in recently weaned pigs (Bilkei *et al.*, 1995).

The clinical expression of PWCC is, to a great extent, affected by herd management (Gyles, 1999). Proper management of the postweaning (PW) period is vital to control PWCC (Bilkei *et al.*, 1996b). Weight and vigour of the piglets, gen-

eral hygiene, environmental temperature, feed supplementation and stocking density in the nursery have to be monitored carefully (Bölcskei *et al.*, 1995).

From the practical point of view PWCC can be subcategorised into postweaning diarrhea (PWD), edema disease (ED), postweaning wasting (PWW) and hemorrhagic gastroenteritis (HGE). Enterotoxigenic *E. coli* (ETEC) is one of the main causes of PWD. The bacterium causes disease by attaching to the intestinal epithelial cells by means of fimbriae and by releasing enterotoxins that reverse the absorption and fluid balance of the small intestine causing hypersecretory diarrhea. Fimbriae (F) 4 and heat – labile (LT) strains are associated with diarrhea. Enterotoxin producing F18 alone or in combination with verotoxin (or "Shiga-like toxin type II.") are responsible for ED and PWW in the majority of cases (Bölcskei *et al.*, 1995). HGE can be caused by ETEC isolates (Bilkei *et al.*, 1995).

Non-pathogenic and pathogenic strains of *E. coli* are commonly found together in the intestinal tracts of weaned piglets (Gyles, 1999). In order to plan preventive measures it is important to identify the virulence factors (Wilson and Francis, 1986). Serologic tests have traditionally been used to identify virulence factors associated with specific clinical symptoms (Moon *et al.*, 1999). Polymerase chain reaction (PCR), - which is now available in many diagnostic laboratories – has markedly increased the rapidity of characterisation of *E. coli* isolates (Bosworth and Casey, 1997). While gene-based tests are technically more demanding than serologic tests, they overcome the problems associated with some virulence determinants under in vitro conditions (Gyles, 1999).

ETEC express two major virulence factors, fimbriae and enterotoxins (Wilson and Francis, 1986). ETEC, pathogenic for swine can express one or more following pilus types: F4, F5, F41, F6, F18 (Bilkei *et al.*, 1995). Verotoxigenic *E. coli* (VTEC or "Shiga-like toxin" producing *E. coli*, SLTEC, Bilkei *et al.*, 1996a) colonise the intestine via the F18 pilus (Moon *et al.*, 1999). In ED, verotoxin causes vascular damage in a variety of organs and the central nervous system (Bilkei *et al.*, 1996b). Pigs suffering from the chronic, subclinical form of ED develop progressing vascular necrosis and wasting (Bilkei *et al.*, 1996b), causing enormous economic losses (Bilkei *et al.*, 1995).

The objective of the present study was to determine the frequency and occurrence of *E. coli* virulence factors and different manifestations of PWCC in a part of eastern Europe with intensive pig production.

MATERIAL AND METHODS

The trial was performed from April to October 2002, in the large "country-corner" of Hungary, Romania and Serbia. From 24 pig production units with 680-1960 inventoried breeding animals per unit, 351 piglets (28-39 days of age), weaned between days 21-28 of lactation were included in the study. They had died with different clinical manifestations of PWCC and were subjected to pathologic-anatomical and PCR assay examination. *E. coli* samples were recovered from the small intestine. Table 1 presents the serogroups and virulence determinants associated with PWCC in these units.

Analysis of the isolates was performed in the laboratory of our consulting office by multiplex PCR assay for detecting heat-labile toxin (LT), heat-stable toxins (STa, STb), enterotoxin (verotoxin, "Shiga-like toxin type II" SLT) and fimbrial adhesins F4 ac, ad, F5 and F18 ab, ac.

In the PCR laboratory procedure as modified by Bosworth and Casey (1997) and Bosworth *et al.* (1998) *E. coli* chromosomal DNA was extracted by boiling, and approximately 100 ng was amplified in 50 ml reactions containing 20 µM dNTPs, approximately 1 µM of each primer (5'-AGGAAGTTATATTTCCGTAGG-3' and 5'-GTATTTGCCTGAACCGTAA-3'), and 1 U Taq polymerase, in 10 mM Tris-HCl (pH8.3), 50 mM KCl, 2 mM MgCl₂. Samples were subjected to 35 PCR cycles, each consisting of 1 min denaturation at 95 °C, 30 sec annealing at 53°C and 30 sec extension at 72 °C. The amplified product was visualised by standard gel electrophoresis of 10 µl of the final reaction mixture on 2% agarose gel. Amplified DNA fragments of specific sizes were located by UV fluorescence after staining with ethidium bromide. The size of the PCR product was read against a digested lambda DNA standard run simultaneously.

The contribution of different manifestations of PWCC such as, postweaning diarrhea (PWD, diarrhea present for 3 days), edema disease (ED, central nervous symptoms, followed by sudden death), postweaning wasting (PWW, marked growth retardation) and hemorrhagic gastroenteritis (HGE, bloody diarrhea and sudden death) was analysed.

RESULTS

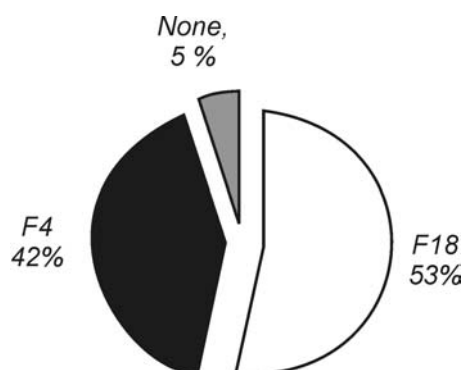
All but four isolates possessed genes for at least one of the enterotoxins or fimbrial adhesins. Isolates that carried fimbrial adhesin genes also carried a gene for at least one of the enterotoxins. The majority (94.87%) of the isolates that carried enterotoxin genes also carried a gene for one of the fimbrial adhesins. The predominant genotypes observed were F4 LT STb and F18 STa STb verotoxin, "Shiga-like toxin type II". (Tables 1,2, Figure 2). Fifty-three percent of the samples showed F18 fimbrial adhesins, 42% F4 virulence factor and 5% none of them (Figure 1).

Table 1. *E. coli* serogroups, virulence factors and diseases present in the investigated units

Serogroup	Fimbrae	Enterotoxin	Disease
O149	F 4 ac, ad	LT, Sta, Stb	Diarrhea
O157:H19	F 4, F 18 ab, ac	LT, Sta, Stb, SLT	Diarrhea, edema disease, wasting
O8	F 4 (and K 99)	LT, Sta, Stb	Diarrhea
O138:K81	F 18 ab, ac	LT, Sta, Stb, SLT	Diarrhea, edema disease, wasting
O139:K82:H1	F 18 ab	LT, Sta, Stb, SLT	Diarrhea, edema disease, wasting
O141:K85:H4	F 18 ac	Sta	Diarrhea, edema disease, wasting

Table 2. Frequency of *E. coli* isolates from pigs suffering from "postweaning coli complex" (PWCC) in units from agricultural Hungary/Romania/Serbia

Fim- brae	No. of stra- ins	Toxins												
		LT	STb	LT STb	Sta STb	LT Sta STb	LT Sta	SLT	SLT STb	SLT LT	SLT LT STb	SLT Sta STb	SLT LT Sta STb	NEG
F 18	187	1	11	16	9	1	2	4	18	2	5	113	4	
F 4	146	1	1	142	1	1								
None	18	1	1	4	3	4	1							4
Total	351	3	13	162	13	6	3	4	18	2	5	113	4	4

Figure 1. Fimbrial adhesins and virulence factors of *E. coli* from pigs suffering from "postweaning coli complex"

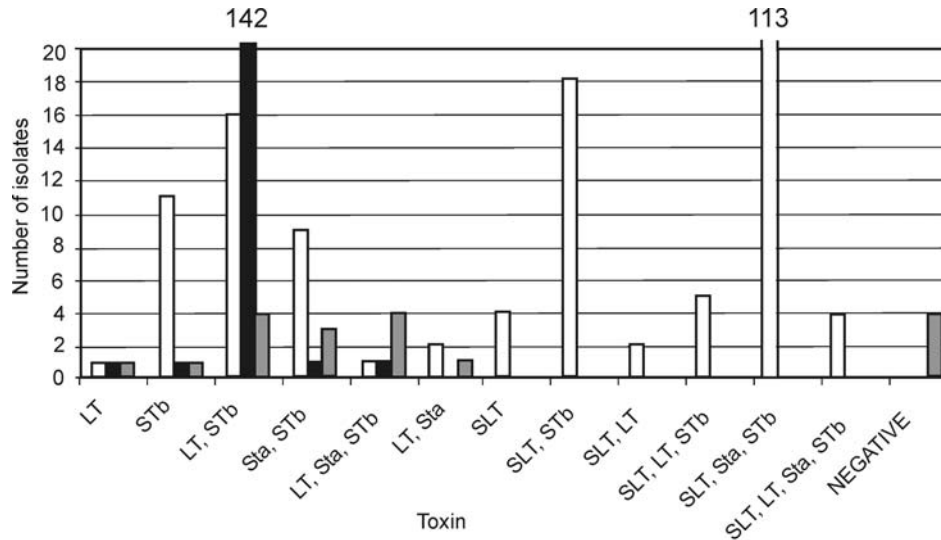


Figure 2. Virulence factors detected in 351 *E. coli* isolates from piglets dying from PWCC (F 18 white, F 4 black, none gray column)

Sixty-nine (20%) animals were diagnosed as having suffered from postweaning diarrhea, thirty-five weaned piglets (10%) edema disease, 240 (68%) postweaning wasting and 7 piglets (2%) hemorrhagic gastroenteritis (Figure 3).

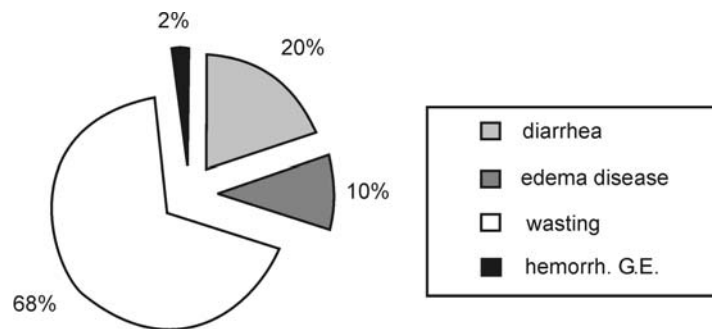


Figure 3. Different manifestations of PWCC from 351 necropsied weaned piglets

DISCUSSION

The results of this survey demonstrate the high frequency of strains expressing F18 fimbriae in the investigated region of Hungary, Romania and Serbia. These strains are typically resistant in vitro to most of the currently approved swine antimicrobials (Bilkei *et al.*, 1995). Since in Eastern Europe there is no currently available commercial vaccine that contains this fimbrial type, most control methods involve dietary management, sanitation measures and the possible oral use of live, nontoxigenic strains to improve local immunity of the gut of the PW piglet (Bölcskei *et al.*, 1995). Such nontoxigenic strains may be naturally harboured in the intestines of PW pigs (Gyles, 1999),

In the present trial the most prevalent fimbrial adhesin was F18. In a recent survey (Moon *et al.*, 1999) F18 has been incriminated in ED and PWW. As F18 fimbriae cannot always be identified in vitro (Witting *et al.*, 1994), a genetic analysis is preferable (Bosworth and Casey, 1997). Gene-based tests overcome the problems associated with some virulence determinants under in vitro conditions. However, PCR does not determine whether a gene is actually encoding a specific virulence factor, only whether a specific segment of DNA is present in the *E. coli* strain being tested. DNA segments from genes containing mutations that render them functionally inactive or that are similar in sequence but different in function, can lead to false positive results.

The PCR technique has been used for more than 10 years in the pig production industry. PCR assays are generally more sensitive than fluorescent antibody tests (FAT), bacterial isolation procedures, immunohistochemistry (IHC) or serological examination. PCR techniques are extremely useful for identifying the presence of specific genomic material of agents and defining genetically controlled characteristics of organisms such as the potential ability to produce toxins.

Consistent with Moon *et al.* (1999) the present data indicate a positive relationship between fimbrial adhesins and enterotoxin genes. In the present study F4 positive *E. coli* usually produced LT and STb. Inconsistent with these authors and the present results Wilson and Francis (1986) stated that F4 – positive *E. coli* produces rather LT, than LT and STb. In Eastern Europe the majority of ED isolates produced F18 SLT or F18 SLT, STb (Bilkei *et al.*, 1995). In the present survey, F18 positive fimbriae were most often found in association with SLT, STa and STb. This increase of genetic combination may be explained by regional clonal expansion.

Eighteen isolates were negative for fimbrial genes. These could be strains that either have lost the capability to produce fimbriae due to loss of plasmid during culture (Wittig *et al.*, 1994), or may suggest a new fimbrial type not currently characterised, or changes in sequence that cause primers to fail to bind.

Consistent with Bilkei *et al.* (1995), in the present survey, the majority of losses included PWW (68%) followed by PWD (20%) and ED (10%). HGE cases (2%) formed a seldomly diagnosed manifestation of PWCC. These results suggest, that – at least in the present trial – the major economic losses may have been caused by subclinical and chronic cases of PWW.

Different possibilities for the control of PWCC are available:

- Immunoprophylaxis might be promising using verotoxin toxoids (Awad-Masalmeh *et al.*, 1989, Johansen *et al.*, 1997)
- Improving social and environmental conditions at weaning (Bilkei *et al.*, 1996b)
- More adequate nutrition of the weaned pigs (Bilkei, 1966b)
- PW zoo- and biotechnique, including the application of antibiotics, probiotics, tripellenamin, melperone, amperozide, clorpromazin, central nervous stimulants, prednisolone or zinc-oxide (Bilkei *et al.*, 1995, Bilkei *et al.*, 1996a,b).

Address for correspondence:
PhD. Dr. med. vet. G. Bilkei
Bilkei Consulting, Raubbühlstrasse 4,
CH – 8600 Dübendorf, Switzerland

REFERENCES

1. Awad-Masalmeh M, Schuh M, Kofer J, Quakyi E, 1989, Verification of the protective effects of toxoid vaccine against edema disease of weaned piglets in an infection model. *Dtsch Tierärztl Wschr*, 96, 419-21.
2. Bilkei G, Biro O, Bölcskei A, Clavadetscher E, Orban P, Waller C, 1995, Practice related management strategies on post – weaning *E. coli* problems in the intensive pig production. Proc. 8th Symposium of the international Society for Animal Hygiene. *Hung Vet J*, 10, 776-7.
3. Bilkei G, Clavadetscher E, Takacs T, Bölcskei A, Kotai I, Uys A, 1996a, Comparison of the effect of Zoo- or combined Zoo- and Biotechnique on the postweaning parameters of the piglets. Proc. 14th IPVS Congress, Bologna, Italy, 7 – 10 July, 266.
4. Bilkei G, Bölcskei A, Clavadetscher E, Biro O, Takacs T, Kotai I, Uys, 1996b, Field trials on early stage of postweaning coli complex oedema disease in intensive pig production units. Proc. 14th IPVS Congress, Bologna, Italy, 7 – 10 July, 257.
5. Bölcskei A, Bilkei G, Biro O, Clavadetscher E, Goos T, Waller C, Stelzer P, 1995, Management der *E. coli* – bedingten Faktorenkrankheiten nach dem Absetzen der Ferkel Berl Münch Tierärztl Wschr, 109, 108 – 11.
6. Bosworth BT, Casey TA, 1997, Identification of toxin and pilus genes in porcine *Escherichia coli* using polymerase chain reaction (PCR) with multiple primer pairs. *Gen Met Am Soc Microbiol B*, 509 (Abstract).
7. Bosworth BT, Dean-Nystrom EA, Casey TA, Neighbors HL, 1998, Differentiation of K88ab from F18 ac *Escherichia coli* by single-stranded conformational polymorphism analysis of the major fimbrial subunit gene 8fedA. *Clin Diagn Lab Immunol*, 5, 299-302.
8. Gyles CG, 1999, Gut edema and the F18 pilus: pathogenesis and laboratory findings. Proc AASP Ann Meet, 457-63.
9. Johansen M, Andersen LO, Jorsal SE, Thomsen LK, Waddel TE, Gyles CL, 1997, Prevention of edema disease in pigs by vaccination with verotoxins 2E Toxoid. *Canadian J Vet Res*, 61, 280-5.
10. Moon HW, Hoffmann LJ, Cornick NA, Boother SL, Bosworth BT, 1999, Prevalences of some virulence genes among *Escherichia coli* isolates from swine presented to a diagnostic laboratory in Iowa. *J Vet Diagn Invest*, 11, 557-60.
11. Wilson RA, Francis DH, 1986. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. *Am J Vet Res*, 47, 213-7.
12. Wittig W, Prager R, Stamm M, Streckel W, Tschape H, 1994, Expression and plasmid transfer of genes coding for the fimbrial antigen in porcine *Escherichia coli* strain. *Zbl Bakt*, 281, 130-9.

FREKVENCA IZOLOVANJA *E. COLI* U MAĐARSKOJ, RUMUNIJI I SRBIJI KOD PRASADI SA RAZLIČITIM MANIFESTACIJAMA KOLI INFEKCIJE POSLE ZALUČIVANJA

DOCIC M i BILKEI G

SADRŽAJ

U ovom radu su prikazani rezultati patomorfoloških nalaza i utvrđivanja prisustva različitih gena za antigene *E. coli* kod zalučene prasadi na gazdinstvima u Mađarskoj, Rumuniji i Srbiji. Dokazivanje antigena je vršeno lančanom reakcijom polimeraze (PCR). Većina izolata je posedovala gene za enterotoksin i adhezine fimbrija. Ukupno je ispitano 351 uzoraka koji su poticali od prasadi sa dijarejom (69), edemskom bolešću (35), mršavljenjem posle odbijanja - zalučivanja (240) i hemoragičnim gastroenteritom (2). Dominantni genotipovi su bili F4 LT STb i F18 STa STb SLT i ovaj nalaz se može tumačiti regionalnom klonskom ekspanzijom.